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An allotriploid derived from a amphidiploid × diploid mating in *Cucumis*

I: Production, micropropagation and verification

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Abstract

A fully fertile interspecific hybrid (*Cucumis hytivus* Chen and Kirkbride, 2n = 4x = 38) between *Cucumis hystrix* Chakr. (2n = 2x = 24) and *C. sativus* L. (2n = 2x = 14) was previously produced by means of F_1 (2n = 19) embryo rescue and subsequent chromosome doubling. This amphidiploid, a new synthetic species, may serve as a genetic bridge in *Cucumis*, and thus is a source for broadening the genetic base of *C. sativus*. The identification and characterization of fertile progeny possessing lower ploidy levels would facilitate bridging among *Cucumis* species. Putative allotriploids (2n = 26) were recovered from *C. hytivus* × *C. sativus* matings by means of embryo culture, and experiments were designed to confirm their genetic constitution, describe their morphology, and establish an efficient protocol for their micropropagation. Apical and axillary buds of these putative allotriploid plants were used as explants to establish a micropropagation system for subsequent verification and characterization of ploidy. Of the array of micropropagation media tested, the most effective for the induction of adventitious buds (desginated Stage II) was a Murashige and Skoog (MS) growth media containing 13.3μM BA + 1.1μM NAA or containing 10 μm BA only. The mean number of adventitious buds per explant in the two media was 6.8 and 6.5, respectively. Shoots resulting from adventitious buds produced roots (Stage III) in relative abundance (39 of 42, 92.8%) on half-strength MS medium containing 1.0 μm IBA. The survivorship of rooted plantlets after acclimatization as assessed by relative production of leaves in plantlets (designated Stage IV) was 91.4% (148 of 162). The chromosome number in putative allotriploid plants as determined in mitotic root tip figures in all plants was 2n = 26, the number expected for allotriploids derived from such a mating. An examination of pollen viability in five samples of each plant by cytochemical staining revealed stainability to be < 10%. Compared to their parents, the allotriploid genotypes possess a high degree of parthenocarpy (84.8%) as measured by setting fruit in pollen-free conditions. While allotriploid fruit are black-spined and similar to the maternal parent *C. hytivus*, the dark green leaves typical of allotriploid plants mirrors that of the paternal *C. sativus* parent.

Abbreviations: BA – N^6-benzyladenine, NAA – α-naphthaleneacetic acid, IBA – indole-3-butyric acid, KT-kinetin, MS-Murashige and Skoog

Introduction

Genetic variation in cucumber (*Cucumis sativus* L., 2n = 14) is relatively limited (Kupper and Staub, 1988). Although the use of wild *Cucumis* species has been suggested for broadening the genetic base of cucumber, strong barriers to interspecific hybridization exist in *Cucumis* (Deakin et al., 1971; Franken et al., 1988). Recently, however, an interspecific hybridization between *C. hystrix* Chakr. (2n = 2x = 24) and *C.*

sativus (Chen et al., 1997a) resulted in the synthesis of a new fertile amphidiploid species *C. hytivus* Chen & Kirkbride (2n = 4x = 38) (Chen et al., 2000). This hybridization represents the uniting of divergent genomes, and thus progeny derived from this amphidiploid may serve as a genetic bridge in *Cucumis* leading to the enhancement of *Cucumis* germplasm. These amphidiploids are in fact cross-compatible with *C. sativus* such that backcrossing to *C. sativus* allows for the introgression of genes from *C. hystrix* (e.g., nematode resistance) at relatively high ploidy levels (2n = 38) (Chen et al., 2002).

It would be desirable to identify and characterize fertile progeny resulting from amphidiploid × *C. sativus* matings that possess chromosome complements more similar to that of *C. sativus*. Allotriploids can have utility in polyploid complexes as bridge genotypes for the production of chromosome addition, substitution and translocation lines that can be employed in chromosome engineering experiments (Barthes & Ricroch, 2001; Zhang et al., 2001). The production of allotripoloids in many species has been difficult, and the resulting allotriploid progeny are generally self-sterile (Hussain et al., 1997a). In preliminary experiments, we obtained some putative allotriploloid embryos as a result of backcrossing *C. hytivus* (amphidiploid, maternal parent) to *C. sativus* (diploid, paternal parent). Nevertheless, the determination of the genetic constitution of putative allotriploid individuals and the subsequent extensive evaluation of genetic variation for economically important traits is resource intensive and requires large plant populations that are difficult to obtain from sterile allotriploids. Thus, it is critical to develop efficient and effective multiplication and maintenance (i.e., cultural methods) protocols for allotriploid utilization. Given the potential significance of allotriploids for improvement of *Cucumis* species and the initial, albeit limited success of allotriploid production from *C. hytivus* × *C. sativus* matings, experiments were designed to characterize putative allotriploid individuals (i.e., cytologically and morphologically) and establish an effective protocol for their micropropagation. The development of such a protocol would allow for the efficient production, identification and propagation of triploid genotypes for use in genetic and breeding studies.

Materials and methods

Production of allotriploid

Seeds of a amphidiploid *C. hytivus* plant and a north Chinese *C. sativus* cultivar Beijing Jietou were germinated in vermiculite. The seedlings were transplanted and matured in a plastic greenhouse at Nanjing Agricultural University between March and September 2000. The *C. hytivus* was used as the maternal parent in controlled hybridization with 'Beijing Jietou' between April and June in 2000 to produce progeny and were later identified. Hybrid fruits were harvested between 20 to 30 days after pollination, and embryos were immediately extracted for culture.

Embryo culture of interspecific hybrids

Fruits were surface-sterilized with 75% ethanol, and seeds were extracted for embryo micro-dissection. Putative allotriploid embryos (i.e., largest embryos observed) were initially identified and selected by visual inspection, and then manually extracted from the integuments with a scalpel. A portion of the embryos were then placed on a basal Murashige & Skoog (1962; MS) medium modified to contain 0.9 μm N^6-benzyladenine (BA) and 0, 10, 30, 60, 90 g l^{-1} sucrose, and sucrose 0, 30 g l^{-1} without BA.Others were transplanted to artificial soil to determine their viability based on emergence. This was designated as Stage I (Establishment). Vigorously growing shoot tips 2 to 4 cm long emerging from developing embryos were used for plantlet micropropagation and culture experiments.

All culture media were adjusted to pH 5.8 prior to the addition of 0.8% (w/v) agar and autoclaving at 125 °C under 104 KPa pressure for 18 min. Treatments (media culture variants) were arranged in a completely randomized design with 8 replicates per treatment. These treatments were then uniformly were incubated at 25 ± 1 °C, under 16-h photoperiod using cool, white fluorescent at an irradiance of 50 μmol m^{-2} s^{-1}. The media and environmental conditions were the same as the following culture stage. The percentage of germinated embryos and the number of shoots formed per explant were recorded. Roots, 1 to 2 cm long, that were derived from the micropropagation of embryos were used for cytogenetic analysis (described below).

Multiplication of explants

Vigorous, 0.5 to 1 cm long shoot explants were selected and transplanted to 150 ml flasks containing 50 ml media containing various BA, BA and NAA combinations in MS medium. This was designated as Stage II (Multiplication). Individual MS media used contained 0, 4.4, 6.7, 11.1, 13.3 μm BA alone or BA 6.7, 11.1, 13.3 μm in combination with NAA 1.1 μm. This factorial set of treatments were arranged in a completely randomized design in which eight replications (flasks), each containing four explants, were cultured per treatment (plant growth regulator concentration). The number of harvestable shoots ($>$ 1 cm) in each treatment was recorded two weeks after placement on each treatment medium.

Rooting and acclimatization of plantlets

Uniform shoots between 1 to 2 cm in length harvested from Stage II cultures were individually separated, and then transferred to rooting media containing half-strength MS basal salts supplemented with 0, 0.5, 2.5, or 5.0 μm IBA. This was designated as Stage III (Rooting). Seven flasks (replicates) containing six shoots each per treatment (IBA concentration) were arranged in a completely randomized design. Rooting percentage [i.e., proportion of roots (longer than 0.5 cm) emerging from plantlet] and the size of calli at the base of the shoots for each treatment was calculated by dividing the number of shoots with roots by the total number of shoots observed.

Well-rooted shoots were rinsed with sterilized water to remove residual rooting media, and then shoots were transferred to 128-plug trays (Agricultural developing company of Lide, Beijing, China) containing a sterilized mixture of sand and soil (3:1 = v:v) for acclimation to greenhouse conditions. This was designated as Stage IV (Acclimatization). To reduce transpiration, a clear 0.05 mm thick polyvinyl plastic cover was placed over each plug tray, and trays were placed in a controlled environment chamber where the irradiance, temperature were the same as that used in Stage I (i.e., tissue culture experiments) and relative humidity was 80%. The plastic cover was gradually removed once a majority of the plantlets exhibited new vegetative growth (about one week). Acclimatized plants (5 to 8 cm in length) were transferred to a greenhouse once the cover had been completely removed ($\sim$ two weeks after initiating acclimatization). The number of plants that survived this acclimatization and transplanting was recorded five days after transplanting.

Characterization of putative allotriploid plants

Morphological observation. Parental plants (*C. hytivus* and *C. sativus*) and acclimatized putative allotriploid plantlets plants obtained from embryo culture (including those for use in cytogenetic analysis) were transplanted to a plastic greenhouse at Nanjing Agricultural University on April 2001.

Five replicates of parental and putative allotriploid plants were compared for the following traits: 1) number of branches; 2) length and diameter of ovary and fruit; 3) spine color (i.e., black or white); 4) leaf color (i.e., reseda or dark green), and; 5) mature fruit color (i.e., gold yellow and straw yellow). The frequency of parthenocarpic fruit development was assessed based the enlargement of unpollinated female flowers (at least 30 flowers) under controlled greenhouse conditions. While means and standard deviations were calculated for metric traits, differences among qualitative traits were assessed by calculating class frequencies by visual examination.

Cytological characterization. Karyotype analysis of chromosomes of roots of germinated embryos and acclimatized plantlets was according to Chen et al. (1998b). The root tips were collected from culture and maintained in cool water (4 °C) for 24 hrs prior to pretreatment for 4 hours in 0.002 m 8-hydroxyquinoline. Root tips were fixed in Carnoy's solution (2 glacial acetic acid: 3 chloroform: 5 ethanol = v:v:v) for 24 hrs, and stored at 4 °C in 70% alcohol until analysis until examination. Root tips were hydrolyzed in 1N HCl for 6 to 10 min at 60 °C, and then stained with acetocarmine. Stained root tips were squashed in 45% acetic acid for metaphase karyotype analysis. At least 30 cells with well-spread chromosomes were observed in each root tip examined. At least 1000 mature pollen grains were stained in 1% aceto-carmine to determine frequencies of pollen stainability according to Momotaz et al. (1998), and photographed using OLYMPUS (BX51) microscope at 500X magnification.

Statistical analysis

Quantitative data were analyzed using ANOVA with treatment means compared using Tukey's HSD test at 0.05 level of probability (Gomez & Gomez, 1984). Qualitative data were calculated and compared as frequencies.

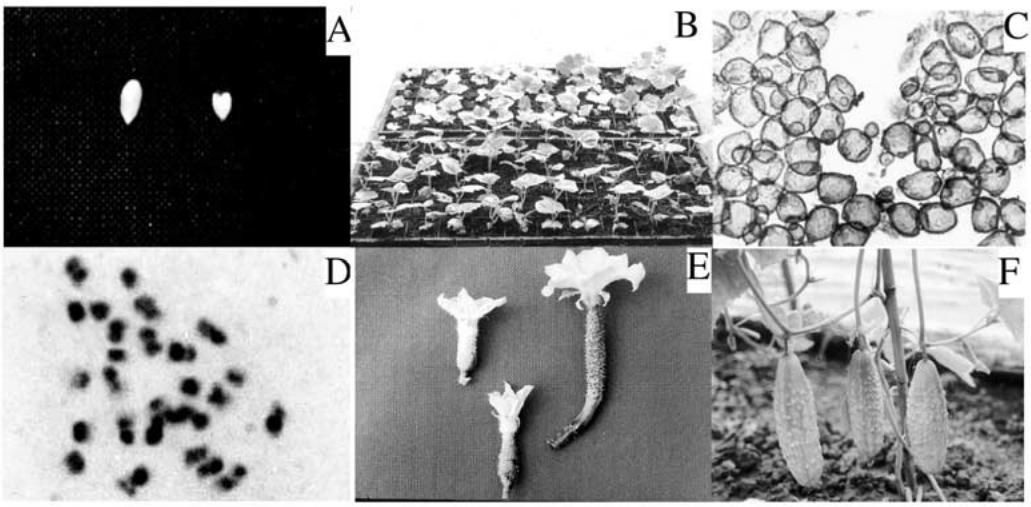

Figure 1. Production and micropropagation of allotriploid plants (3n = 26) derived from adventitious buds recovered from a *C. hytivus* Chen & Kirkbride (2n = 4x = 38) × *C. sativus* L. (2n = 2x = 14) mating. Panel A: Seed of cultivated cucumber cv. Beijing Jieou (left side) and allotriploid germplasm (right side). Panel B: Allotriploid plants two weeks after transplantation into a plug tray containing sand and soil (3:1; v:v) in preparation for acclimatization in a greenhouse. Panel C: Pollen grain shapes produced by allotriploid plants. Panel D: Metaphase chromosomes typical of allotriploid (2n = 26) plants. Panel E: Female flowers from an synthetic *Cucumis* amphidipoid (*C. hystivus*; upper left), a diploid cucumber 'Beijing Jietou' (*C. sativus*; upper right) and a *Cucumis* allotriploid (lower middle). Panel F: Typical fruit of *Cucumis* allotriploid genotypes.

Results

Embryo culture of interspecific hybrids

Pollination of 20 pistillate flowers of *C. hytivus* plants by *C. sativus* L. *cv.* Beijing jietou produced 14 fruits (i.e., 70% fruit set). One hundred and twenty six embryos (> 3 mm in length) were recovered from these fruits (i.e., on average, 8.9 seed per fruit). Ninety-six embryos were selected randomly for rescue experiments leading to plantlet propagation, and the remaining 30 embryos were transplanted to soil for an assessment of vigor and emergence. All 30 immature embryos failed to germinate (Figure 1, Panel a).

Of the 96 embryos, fifty-two germinated on Stage I medium. With 0.2 mg l^{-1} BA, higher sucrose levels (> 30 g l^{-1}) resulted in a relatively higher embryo germinating percentages when compared to the control treatment (MS media without BA). The germination rates were 53.3%, 80.0%, 86.6% as the sucrose concentration were 30, 60, 90 g l^{-1} respectively, while only 15.4% when without sucrose and BA. These results recapitulated those of Chen et al. (1997).

Multiplication of explants

Data indicate that growth of explants in Stage II (i.e., induction of adventitious buds) was enhanced by their culture on MS medium supplemented with 10 μm BA only (Table 1). This media supplemented with 10 μm BA resulted in the production of explants with a great number of uniform and vigorous shoots per explants (average/explant = 6.5). Shoot production from adventitious buds decreased when propagules were grown on MS media containing BA concentrations higher than 11.1 μM. Moreover, shoots yellowed and developed poorly when adventitious buds were grown on MS media when BA concentrations reached to 20 μm. Likewise, shoot differentiation from apical

Table 1. Effect of BA concentration on the induction of adventitious bud derived from a *Cucumis hytivus* × *C. sativus* L. mating

BA concentration (μM)	Number of shoots per explant[1]	Shoot length (cm)[1]
0	1.46 ± 0.23^{a2}	2.22 ± 0.40^{a}
4.4	3.90 ± 0.54^{b}	1.58 ± 0.28^{ab}
6.7	3.72 ± 0.29^{b}	1.29 ± 0.25^{b}
10	6.53 ± 0.50^{c}	0.94 ± 0.14^{b}
11.1	5.00 ± 0.91^{bc}	1.07 ± 0.33^{b}
13.3	4.13 ± 0.61^{b}	1.00 ± 0.23^{b}

[1] Evaluation was made after 15 days in culture.
[2] Means followed by the same letters are not significantly different ($p < 0.05$) by Tukey's test.

Table 2. The effects on IBA concentration nutrition on rooting of adventitious bud explants derived from a *Cucumis hytivus* × *C. sativus* L. mating

Media for rooting	Rooting ratio[1] (%)	Calli (cm)[2]
MS	45.8	0.23 ± 0.1
MS + IBA 1.0 μM	64.6	0.53 ± 0.2
MS + IBA 2.5 μM	81.2	0.63 ± 0.1
1/2 MS	60.4	0.27 ± 0.2
1/2 MS + IBA 1.0 μM	92.8	0.15 ± 0.1
1/2 MS + IBA 2.5 μM	89.6	0.73 ± 0.1
1/2 MS + IBA 5.0 μM	97.9	0.97 ± 0.2

[1] Proportion of roots (longer than 0.5 cm) emerging from plantlet was recorded 10 days later since shoots were transplanted on rooting media.
[2] Diameter of calli were evaluated at the base of plantlets at the point of insertion of shoots into calli, and means and standard deviations were determined on the plantlets evaluated.

buds was less than two per explants when propagules were grown on MS media containing 4.4 μM BA.

In general, shoots from explants grown on MS media containing both NAA and BA were shorter and more uniform than those that developed on plant growth regulator free media or containing BA only. In BA combination with NAA media, shoot differentiation and growth from apical buds was greatest on MS media containing 13.3 μM BA + 1.1 μM NAA, and the number of uniform and vigorous shoots per explants was up to 6.8 (average/explant = 6.8) (data not shown).

The growth of embryos in culture of derived from interspecific mating was more rapid than that of *C. hytivus* and cultivated cucumber (data not shown). Moreover, the shoots of putative allotriploid plantlets grew to between 3 to 4 cm in length in two weeks, and thus required transfer to other media in order to prevent leaf yellowing and tissue deterioration. This transfer occurred two weeks earlier that parental lines.

Rooting and acclimatization of plantlets

Well-developed shoots were excised from *in vitro* plants grown in Stage II, and then these propagules were cultured on MS medium with or without IBA (Table 2). On average, four roots per shoot developed within 10 days when cultured on half-strength MS medium. The rooting medium consisting of half-strength MS medium supplemented 1.0 μm IBA resulted in the highest frequency of rooting (92.8%), and subsequently the highest degree of shoot acclimatization. It was observed that culture at relatively high concentrations of IBA (> 2.5 μM) resulted in greater callus formation when compared to media free or at lower concentrations of IBA. The plantlets from theses media containing more 2.5 μm resulted in decreased acclimatization and survival (data not presented). Plantlets recovered from Stage III grew vigorously under controlled growth chamber conditions (Figure 1, panel b), resulting in 91.4% plantlet survivorship (148 of 162 plantlets). After two weeks, plantlets were successfully transferred to soil, after which their growth was normal and uniform.

Cytogenetic and morphological characterization of putative allotriploid plants

Cytogenetic examination. The chromosome number can be used for unequivocally identifying hybrids when the parents possess different chromosomes (Takatsu et al., 2001). The chromosome number of amphidiploid *C. hytivus* and *C. sativus* L. *cv.* Beijing jietou is 38 and 14, respectively. The chromosome number of interspecific hybrids resulting from a mating between these individuals would be expected to be 2n = 3x = 26. This prediction was confirmed by chromosome counts employing root tip meristematic cells recovered from putative allotriploids (Figure 1, panel d).

Pollen stainability of samples taken from selected plants was remarkably less than those of either parents (Table 3). Pollen grain stainability tests of these putative allotriploids indicated that only 10% of the pollen grains examined absorbed the staining solution. These plants were likely male sterile given the lack of stain absorption in many pollen grains, and the varying and irregular shape and size of stained pollen grains in these selections (Figure 1, panel c).

Morphological characterization. Putative allotriploid plantlets were initially identified by their unique morphology (Table 3; Figure 1). Acclimatized plantlets matured rapidly, were uniform (i.e., no somaclonal variation), and possessed characteristics common to both their *C. hytivus* and *C. sativus* parents (Table3). While fruit weight, number of branches, ovary length, fruit length and spine (i.e., black) and mature fruit (i.e., golden) of progeny more closely resembled their maternal *C. hytivus* parent (Figure 1, panels e and f), developmental vigor (i.e., relative growth rate) and leaf color (i.e., dark green vs. reseda of *C. hytivus*) was more similar to and characteristic of the paternal,

Table 3. Morphological characteristics of *Cucumis hytivus* (2n = 4x = 38), *C. sativus* L. cv. Beijing jietou (2n = 2x = 14), and allotriploid plants (3n = 3x = 26) derived from a *C. hytivus* × *C. sativus* mating

Traits[1]	*Cucumis hytivus*	Allotriploid plantlets	*Cucumis sativus* Beijing jietou
Average number of branches	4.6 ± 1.9[2]	4.9 ± 1.2	1.3 ± 0.6
Ovary length (cm)	1.3 ± 0.2	1.9 ± 0.2	5.4 ± 0.70
Average fruit length (cm/fruit)	7.5 ± 1.4	12.1 ± 2.0	51.7 ± 3.5
Average fruit weight (gm/fruit)	35.4 ± 4.3	123.2 ± 5.8	322.4 ± 14.4
Mean pollen grain stainability (%)	21.9 ± 2.6	9.8 ± 2.50	94.4 ± 4.28
Fruit length:diameter ratio	2.2 ± 0.3	3.4 ± 0.2	12.5 ± 0.9
Parthenocarpic rate (%)	26.7	84.8	45.2
Fruit spine color	Black	Black	White
Leaf color	Reseda	Dark green	Dark green

[1] Primary branch number was recorded before season-end vegetative decline; ovary length and pollen grain stainability were recored at anthesis; five mature fruits were used for the determination of fruit weight, length and diameter fruit index, and spin color and leaf color was observed during the vegetative growth period.
[2] Data are shown with mean ± SE.

C. sativus, parent. Progeny were more parthenocarpic than either parent bearing abundant seedless fruit (i.e., 84.8% developed fruit under greenhouse conditions). While their parents *C. hytivus* and *C. sativus* were lower (26.7% and 45.2% respectively) (Table 3).

Discussion

Based on morphological and cytogenetic analysis, plants obtained from the culture of embryos derived from a *C. hytivus* × *C. sativus* were classified as allotriploid (2n = 3x = 26). Their ploidy level was predictable based on previous cross-compatibility and cytogenetic examination of *C. sativus* (Chen et al., 1998), and *C. hytivus* (Chen et al., 2002). This is the first report of the recovery of an allotriploid in *Cucumis* species derived from a mating between commercial cucumber and a synthetic amphidiploid. Allotriploid plants were recovered via embryo rescue, which was facilitated by the identification of culture media, and protocols that enhanced the development of plantlets from embryos.

The defined protocol for multiplication presented herein enables the timely synthesis and recovery of a relatively large number of genetically identical allotriploid plants for both research and commercial production. The allotriploid plants obtained from micropropagation are uniform and possess unique characteristics that include sequential fruit set and tolerance for growth under low temperature and light (Chen et al., 2002). The length/diameter ratio of parthenocarpic fruit recovered from allotriploid plants was 3.4. In China, and perhaps other countries, such allotriploid plants have potential for introduction as alternative germplasm for its pickling industry. However, frequent subculture of the plant may reduce its proliferating capacity and increase its genetic variation (e.g., somaclonal variation). Therefore, it would be critical to develop techniques, which preserve the genetic constitution of original allotriploid individuals during long-term culture.

Allotriploid plants can produce unreduced pollen that possesses a 2n chromosome complement (Hou et al., 1997). Plants which produce unreduced pollen when used in conjunction with genetic engineering technologies such as alien addition, substitution and translocation lines in repetitive backcrossing strategies can allow for the introgression of foreign genes into commercial cultivars (Barthes and Ricroch 2001). It is possible that genetic bridges between *Cucumis* species [i.e., *C. sativus* and *C. melo* L. (melon; 2n = 2x = 24)] can be attained through continued development and strategic application of genetic stocks derived from the allotriploid germplasm described herein. Transfer of economically important genes (e.g., nematode resistance, growth under low irradiance) from the synthetic amphidiploids (Chen et al., 2002) or wild African species (Staub et al., 1992) could ultimately broaden the narrow germplasm base of cucumber and melon (Horejsi and Staub, 1999; Staub et al., 2000)

and result in enchanced germplasm for commercial production.

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References

Barthes, L. and A. Ricroch, 2001. Interspecific chromosomal rearrangements in monosomic addition lines of *Allium*. Genome 44: 929–935.

Chen, J., J.E. Staub, Ch. Qian, J. Jiang, X. Luo, and F. Zhuang, 2002. Reproduction and cytogenetic characterization of interspecific hybrids derived from *Cucumis hystrix* Chakr. × *C. sativus* L. Theor Appl Genet (in press).

Chen, J.F. & J.H. Kirkbride, Jr., 2000. A new synthetic species *Cucumis* (Cucurbitaceae) from interspecific hybrridization and chromosome doubling. Brittonia 52: 315–319.

Chen, J.F., J.E. Staub, Y. Tashiro & S. Miyazaki, 1997. Successful interspecific hybridization between *Cucumis sativus* L. and *C. hystrix* Chakr. Euphytica 96: 413–419.

Chen, J.F., J.E. Staub & J. Jiang, 1998. A reevaluation of karyotype in Cucumber (*Cucumis sativus* L.). Genet Res Crop Evol 45: 301–305.

Chen, Q., J. Jahhier & Y. Cauderon, 1992. Production and cytogeneetic analysis of BC_1, BC_2, and BC_3 progeneies of an intergenetic hybrid between *Triticum aestivum* (L.) Thell and tetraplopid *Agropyron cristatun*. Theor Appl Genet 84: 698–703.

Deakin, J.R., G.W. Bohn & T.W. Whitaker, 1971. Interspecific hybridization in *Cucumis*. Econ Bot 25: 195–211.

Franken, J., J.B.M. Custers & R.J. Bino, 1988. Effects of temperature on pollen tube growth and fruit set in reciprocal crosses between *Cucucmis sativus* and *C. metuliferus*. Plant Breeding 100: 150–153.

Gomez, K.A. & A.A. Gomez, 1984. Statistical procedures for agricultural research. 2nd edn. John Wiley, NY.

Horejsi, T. & J.E. Staub, 1999. Genetic variation in cucumber (*Cucumis sativus* L.) as assessed by random amplified polymorphic DNA. Genet Res Crop Evol 46: 337–350.

Hou, L.X., W.J. Li & H.Y. Zhou, 1997. Study on the formation and utilization of 2n pollen of closely related triploid hybrids of sweet potato. J China Agric Univ 2: 91–99 (in Chinese with English summary).

Hussain, S.W., W.M. Williams, D.R. Woodfield & J.G. Hampton, 1997. Development of a ploidy series from a single interspecific *Trifolium repens* L. × *T. nigrescens* Viv. F_1 hybrid. Theor Appl Genet 94: 821–831.

Kupper, R.S. & J.E. Staub, 1988. Combining ability studies between lines of *Cucumis sativus* L. and *C. sativus* var. hardwaickii (R.) Alef. Euphytica 38: 197–216.

Momotaz, A., M. Kato & F. Kakihara, 1998. Production of intergeneric hybrids between *Brassica* and *Sinapis* species by means of embryos rescue techniques. Euphytica 103: 123–130.

Murashige, T. & F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497.

Staub, J.E., L.D. Knerr, D.J. Holder & B. May, 1992. Phylogenetic relationships among several African *Cucumis* species. Can J Bot 70: 509–517.

Staub, J.E., Y. Danin-Poleg, G. Fazio, T. Horejsi, N. Reis & N. Katzir, 2000. Comparative analysis of melon (*C. melo* L.) germplasm using random amplified polymorphic DNA and simple sequence repeat markers. Euphytica 115: 225–241.

Takatsu, Y., M. Kasumi, T. Manabe & M. Hayashi, 2001, Temperature effects on interspecific hybridization between *Gladiolus* × *grandiflora* and *G. tristis*. HortScience 36 (2): 341–343.

Zhang, C.H, S.L. Zhang, X. Shen, M. Wang & Y.H. Wang, 2001. Observation on obtaining the triploid by $4rmx \times 2x$ and its cytoembryology in false pakchoi. Acta Hort Sin 28: 317–322.